Clinical Implications of Circulating Tumor DNA in Multiple Myeloma and Its Precursor Diseases

Sung-Soo Park, M.D., Ph.D.[1,2*], Na Yung Kim, M.S.[3*], Ji-Young Lim, Ph.D.[1,2], Jung Yeon Lee, M.D.[1,2], Sujin Yun, B.S.[3], Yeun-Jun Chung, M.D., Ph.D.[3,4,5], Seung-Hyun Jung, Ph.D.[3,5,6], and Chang-Ki Min, M.D., Ph.D.[1,2]

[1]Department of Hematology, Seoul St. Mary's Hematology Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea; [2]Catholic Research Network for Multiple Myeloma, Catholic Hematology Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea; [3]Department of Medical Sciences, Graduate School of The Catholic University of Korea, Seoul, Korea; [4]Department of Microbiology, College of Medicine, The Catholic University of Korea, Seoul, Korea; [5]Catholic Research Institute for Human Genome Polymorphism, Precision Medicine Research Center, College of Medicine, The Catholic University of Korea; Seoul, Korea; [6]Department of Biochemistry, College of Medicine, The Catholic University of Korea, Seoul, Korea

Supplemental Data

Supplemental methods

Extraction of circulating tumor DNA (ctDNA) and preparation of bone marrow (BM) for next-generation sequencing (NGS)

A 10-mL aliquot of whole peripheral blood (PB) collected in an ethylenediamine tetraacetic acid tube was centrifuged at 1,600×*g* for 10 min within 2 hr of sample collection. The separated plasma was transferred to a new 1.5-mL tube, followed by centrifugation at 16,000×*g* for 10 min. DNA was extracted from 1 mL of plasma using a MagMAX™ Cell-Free DNA Isolation Kit (Thermo Fisher Scientific, Waltham, MA, USA). ctDNA concentrations were measured using a Qubit 3.0 fluorometer and Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific). Plasma ctDNA concentrations were calculated assuming a plasma volume of 1 mL.

To purify plasma cells from BM, BM cells were mixed with anti-CD138 microbeads (cat. No. 130-051-301; Miltenyi Biotec, Bergisch Gladbach, Germany) and loaded onto an LS column (Miltenyi Biotec). The magnetically labeled $CD138^+$ cells were eluted and used as plasma cells. DNA was extracted from the $CD138^+$ plasma cells using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany).

Targeted NGS and identification of somatic variants

We performed targeted NGS, as previously described [1]. Briefly, OncoChase cancer panels targeting 156 cancer-related genes (ConnectaGen, Seoul, Korea) were used to generate sequencing libraries (Supplemental Data Table S1). Sequencing libraries were generated using an AmpliSeq Library Kit 2.0 (Thermo Fisher Scientific) and sequenced using the Ion S5 system (Thermo Fisher Scientific), according to the manufacturer's instructions. Sequencing reads were aligned to UCSC hg19, and genomic variants were detected using

Torrent Suite v5.12.1. The ANNOVAR package [2] was used to select somatic variants located in exonic sequences and predict their functional consequences. Stringent post-filtering was conducted to ensure reliable and robust mutation calling. First, known polymorphic sites in East Asians (>0.1% of minor allele frequency) in public databases (dbSNP137, ESP6500, and the 1000 Genomes Project) were filtered out as germline polymorphisms. Subsequently, variants with a total read depth <50 or variant support read depth <3 were filtered. The remaining variants were considered somatic mutations. Only non-silent mutations were used to compare quantitative and qualitative differences in (driver) mutations among disease groups. The goal of serial ctDNA profiling was to analyze minimal residual disease (MRD) based on somatic mutations in tumor cells; therefore, silent mutations were also monitored.

DNA copy number analysis

DNA copy number alterations (CNAs) were estimated using the targeted NGS data. We used targeted NGS data from normal circulating free DNA collected from seven healthy donors as a pooled reference. CNAs were identified using Ion Reporter v5.12.2 (Thermo Fisher Scientific). Samples with a median absolute pairwise difference ≥0.4 (N=25) were excluded from the CNA analysis. CNAs with confidence scores ≤ 10 were also excluded. Segments were classified as copy number gains or losses when their ploidy was ≥3 and ≤ 1 N, respectively. All CNAs identified were manually curated based on the sequencing depth ratio.

Subcohort selection to explore the trajectory of ctDNA during disease course

Among 89 patients in the MM cohort, we selected the following subcohorts: (i) two patients (case Nos. MM510 and MM429) who had serial samples available after the initiation of frontline treatment, with additional samples obtained when they achieved sustained MRD

negativity; (ii) five patients (case Nos. MM564, MM347, MM514, MM543, and MM233) who relapsed after achieving very good partial response or better following frontline treatment, with samples provided at each time point of relapse. For patients receiving daratumumab monotherapy (case No. MM233), monthly serial samples were obtained as per the established prospective protocol. Response criteria and MRD detection methods are described in previous reports [3, 4].

Statistical analysis

We used the chi-squared or Fisher's exact test to compare categorical variables. To compare continuous variables among the three groups, we used one-way analysis of variance or the Kruskal–Wallis test, depending on whether the normality assumption of each variable was satisfied, as determined by the Shapiro-Wilk test. Correlations between ctDNA mutations and clinical features were analyzed using Pearson's correlation. For the comparison of continuous values, we used the Mann–Whitney U-test or Student's *t*-test. We performed logistic regression analyses to identify parameters related to MM-related presentation. Parameters with $P < 0.05$ on the Student's *t*-test or chi-squared test were included as covariates. Survival outcomes, including PFS and OS, were estimated using the Kaplan–Meier method with a log-rank test. PFS was defined as the time from the date of frontline treatment to any event for PFS or the date of the last follow-up. Events for PFS were disease progression and death, and OS was defined as the time from the initiation of frontline treatment to death from any cause or the date of the last follow-up. We performed Cox proportional hazards regression analysis using variables with $P < 0.05$ on univariate analysis as covariates. $P < 0.05$ was considered statistically significant in all analyses. Statistical analyses were performed using the Statistical Package for the Social Sciences v25 (IBM, Armonk, NY) and R.

Supplemental Data Table S1. Target genes in the OncoChase cancer panel

OncoChase target genes (N=156)						
MTOR	*ARID1A*	*CSF3R*	*MPL*	*JAK1*	*NRAS*	*TENT5C (FAM46C)*
MCL1	*DDR2*	*MDM4*	*MYCN*	*DNMT3A*	*ALK*	*MONO27*
BAT26	*XPO1*	*NR24*	*THSD7B*	*LRP1B*	*PKP4*	*NFE2L2*
SF3B1	*IDH1*	*ERBB4*	*SP140*	*GRM7*	*VHL*	*RAF1*
MLH1	*MYD88*	*SCN11A*	*CTNNB1*	*RHOA*	*FOXP1*	*ABI3BP*
PLD1	*PIK3CA*	*ZNF595*	*FGFR3*	*PDGFRA*	*KIT*	*BAT25*
KDR	*TET2*	*FBXW7*	*TERT*	*SPEF2*	*CHD1*	*APC*
EGR1	*CSF1R*	*NPM1*	*FGFR4*	*IRF4*	*PRDM1*	*ROS1*
ESR1	*MLLT4 (AFDN)*	*RAC1*	*IKZF1*	*EGFR*	*CDK6*	*MET*
SMO	*BRAF*	*EZH2*	*KMT2C*	*NKX3-1*	*FGFR1*	*MYC*
JAK2	*CD274 (PD-L1)*	*PDCD1LG2 (PD-1)*	*CDKN2A*	*GNAQ*	*ZNF462*	*ABL1*
NOTCH1	*ANKRD26*	*PTCHD3*	*RET*	*PTEN*	*PLEKHS1*	*FGFR2*
PTPRE	*HRAS*	*OR5L1*	*WDR74*	*CCND1*	*FAT3*	*NR27*
ATM	*SDHD*	*CDKN1B*	*KRAS*	*KMT2D (MLL2)*	*ERBB3*	*CDK4*
MDM2	*PTPN11*	*POLE*	*PARP4*	*FLT3*	*BRCA2*	*RB1*
DIS3	*NR21*	*FOXA1*	*RAD51B*	*TSHR*	*C14orf49 (SYNE3)*	*TRAF3*
AKT1	*NIPA2*	*B2M*	*MAP2K1*	*IDH2*	*CYLD*	*CDH1*
ZFHX3	*FANCA*	*USP6*	*TP53*	*MYH13*	*NF1*	*CDK12*
ERBB2	*IKZF3*	*BRCA1*	*SPOP*	*SRSF2*	*SETBP1*	*SMAD4*
STK11	*GNA11*	*MAP2K2*	*CALR*	*JAK3*	*CCNE1*	*CEBPA*
RYR1	*ASXL1*	*SRC*	*PTPRT*	*AURKA*	*GNAS*	*RUNX1*
U2AF1	*MAPK1*	*DDX17*	*KDM6A*	*ARAF*	*AR*	*MED12*
BRWD3	*RPL10*					

Background color indicates genes reported to be frequently mutated in multiple myeloma [5].

Supplemental Data Table S2. Description of the targeted deep sequencing data

No. case	ctDNA concentration (ng/mL)	Mapped reads (N)	On-target (%)	Mean depth	Uniformity (%)
MGUS1	10.8	4,591,377	95.97	2,287	94.10
MGUS2	85.5	2,538,746	93.95	1,229	93.77
MGUS3	199.5	5,243,708	96.04	2,613	95.44
MGUS4	8.6	2,692,335	95.11	1,312	83.72
MGUS5	6.5	3,466,322	96.06	1,723	93.89
MGUS6	9.7	5,628,975	95.89	2,795	94.40
MGUS7	12.2	2,932,385	96.33	1,463	94.12
SMM1	13.1	4,069,471	96.10	2,026	94.80
SMM2	11.7	4,595,132	96.13	2,269	94.07
SMM3	10.0	1,535,535	95.67	1,199	95.70
SMM4	9.1	4,722,796	96.05	2,344	94.03
SMM5	8.8	5,035,622	96.70	2,512	87.06
SMM6	1.9	5,476,055	96.49	2,740	93.84
MM01	225.0	2,291,492	93.25	1,103	90.42
MM02	17.9	2,431,088	94.43	1,183	94.67
MM03	150.0	3,475,885	94.90	1,704	94.66
MM04	3.5	5,248,536	95.61	2,584	94.31
MM05	12.0	4,674,988	96.51	2,340	91.56
MM06	17.3	3,274,402	96.07	1,627	93.26
MM07	3.9	6,092,450	96.52	3,045	92.83

MM08	21.5	5,008,482	94.03	2,434	95.03
MM09	3.7	4,238,441	93.62	2,042	84.62
MM10	4.3	6,034,689	96.32	3,020	92.97
MM11	33.0	4,956,083	94.57	2,425	92.80
MM12	8.6	1,621,091	94.45	1,251	94.56
MM13	24.2	3,338,590	96.29	1,665	93.39
MM14	18.8	3,381,499	96.08	1,677	93.59
MM15	4.9	4,827,181	96.21	2,397	93.74
MM16	7.3	4,940,216	96.25	2,457	94.49
MM17	3.5	5,923,682	96.25	2,959	94.07
MM18	118.9	4,038,197	94.55	1,973	95.28
MM19	30.9	1,808,565	87.72	1,225	83.16
MM20	285.0	2,435,849	92.08	1,158	91.40
MM21	132.8	4,520,604	96.40	2,253	94.88
MM22	5.2	3,410,300	83.33	1,372	73.98
MM23	5.9	4,526,223	95.83	2,240	94.56
MM24	523.5	4,190,530	93.72	2,028	94.88
MM25	298.4	3,296,326	92.63	1,567	94.29
MM26	98.4	4,459,401	93.20	2,138	90.29
MM27	9.6	4,964,562	95.67	2,448	94.90
MM28	52.8	4,231,178	96.46	2,114	73.99
MM29	111.4	4,070,181	95.84	2,019	94.95
MM30	9.6	1,733,196	95.75	1,345	94.57
MM31	2.8	5,598,537	97.13	2,821	90.82
MM32	92.5	5,584,695	94.10	2,712	92.97

MM33	6.4	4,152,749	94.35	2,033	89.03
MM34	18.3	3,212,602	92.02	1,511	84.77
MM35	108.0	6,020,680	93.86	2,916	94.60
MM36	17.6	4,271,142	94.76	2,089	94.87
MM37	83.4	1,583,862	95.25	1,212	94.61
MM38	5.9	5,660,620	96.15	2,814	93.88
MM39	50.8	4,971,416	94.34	2,424	86.20
MM40	7.3	4,124,163	95.94	2,048	94.12
MM41	32.0	1,111,995	81.47	674	69.14
MM42	35.6	4,288,209	94.16	2,095	92.62
MM43	3.1	5,457,888	96.85	2,750	91.73
MM44	21.3	3,665,391	96.26	1,821	93.77
MM45	14.5	4,220,830	94.13	2,056	95.11
MM46	10.2	3,020,406	95.92	1,498	94.38
MM47	12.3	4,216,694	95.01	2,092	67.34
MM48	6.5	3,784,156	91.01	1,743	79.85
MM49	27.6	4,627,332	94.44	2,250	94.61
MM50	28.2	6,028,102	93.70	2,910	95.26
MM51	60.6	3,273,640	93.70	1,582	89.50
MM52	36.8	1,129,591	82.06	679	66.58
MM53	24.2	3,814,296	95.93	1,881	94.30
MM54	10.2	4,574,892	95.78	2,249	94.22
MM55	58.2	4,704,614	94.72	2,318	53.28
MM56	8.6	4,383,528	95.71	2,162	93.97
MM57	5.8	5,380,307	96.18	2,667	94.05

MM58	9.0	4,503,125	96.47	2,247	94.35
MM59	5.4	3,708,844	96.02	1,836	94.49
MM60	48.3	6,016,186	95.32	2,961	95.54
MM61	15.3	5,053,464	96.36	2,517	93.74
MM62	5.5	5,288,912	93.28	2,547	89.54
MM63	5.8	4,577,754	95.72	2,266	94.24
MM64	3.5	4,900,682	96.36	2,445	93.54
MM65	6.2	4,310,791	94.28	2,109	88.47
MM66	16.6	5,707,824	93.67	2,752	94.94
MM67	254.4	3,542,658	92.92	1,691	94.39
MM68	187.0	4,565,464	94.64	2,227	94.46
MM69	17.0	3,815,211	94.55	1,863	94.80
MM70	18.2	3,402,674	95.88	1,684	93.64
MM71	6.2	5,387,550	96.33	2,689	93.80
MM72	118.4	4,571,850	95.97	2,278	94.93
MM73	9.2	5,116,742	95.82	2,528	94.36
MM74	6.2	5,146,862	96.37	2,579	93.81
MM75	6.8	4,566,411	94.20	2,226	88.08
MM76	8.4	1,662,481	95.35	1,296	94.66
MM77	9.1	4,752,007	96.26	2,356	93.57
MM78	6.1	4,980,008	95.95	2,478	94.35
MM79	13.5	5,094,909	96.12	2,535	94.21
MM80	4.6	4,743,001	96.02	2,351	94.41
MM81	6.2	5,500,486	95.88	2,734	94.45
MM82	351.8	4,152,197	93.55	2,007	95.39

MM83	36.3	4,450,004	93.70	2,164	92.46
MM84	70.4	5,046,992	94.56	2,470	93.65
MM85	11.8	2,062,073	96.21	1,027	93.66
MM86	51.4	4,309,090	88.95	1,989	52.70
MM87	10.7	4,341,112	96.51	2,160	91.69
MM88	34.4	3,027,091	90.95	1,422	72.09
MM89	4.8	5,475,312	95.94	2,715	94.24

Supplemental Data Table S3. Somatic point mutations and indels identified in 102 ctDNA genomes

The contents of Supplemental Data Table 3 are provided in a separate Excel file.

Supplemental Data Table S4. Copy number alterations identified in 11 patients with at least one copy number alteration

No. case	Gene	Position	Event
MGUS7	*MYC*	chr8:128746883-128756332	Gain
MM03	*MCL1*	chr1:150547812-204518488	Gain
MM03	*NIPA2*	chr15:23006630-90645638	Gain
MM03	*TET2*	chr4:106067077-153332977	Gain
MM06	*CCND1*	chr11:69462876-111957573	Gain
MM06	*FOXP1*	chr3:71101701-178952165	Gain
MM07	*NIPA2*	chr15:23006630-23021287	Gain
MM25	*NKX3-1*	chr8:23534856-128756332	Gain
MM32	*MTOR*	chr1:11168251-11319360	Gain
MM32	*NRAS*	chr1:115247335-118166394	Loss
MM32	*FLT3*	chr13:28636019-32972922	Loss
MM32	*NIPA2*	chr15:23006630-66774204	Gain
MM32	*TP53*	chr17:7577486-7579950	Loss
MM32	*STK11*	chr19:1206878-13050094	Gain
MM32	*FGFR3*	chr4:1795711-55589817	Loss
MM32	*APC*	chr5:112090645-112116546	Gain
MM46	*MYC*	chr8:128746883-128756332	Gain
MM59	*MCL1*	chr1:150547812-204518488	Gain
MM61	*STK11*	chr19:1206878-4123905	Gain
MM65	*MYC*	chr8:128746883-128756332	Gain
MM68	*MCL1*	chr1:150547812-150552208	Gain
MM68	*MDM4*	chr1:204494635-204518488	Gain
MM68	*RB1*	chr13:49039304-73350105	Loss

Supplemental Data Table S5. Gene alteration numbers and frequencies

Gene	Total cohort (N=102)		MGUS (N=7)		SMM (N=6)		MM (N=89)		*P*
	Alteration		Alteration		Alteration		Alteration		
	N	%	N	%	N	%	N	%	
KRAS	15	14.7	0	0.0	0	0.0	15	16.9	0.546
NIPA2	13	12.7	0	0.0	2	33.3	11	12.4	0.205
TP53	11	10.8	0	0.0	0	0.0	11	12.4	1.000
GNAS	11	10.8	1	14.3	2	33.3	8	9.0	0.096
ZFHX3	9	8.8	1	14.3	0	0.0	8	9.0	0.722
NRAS	9	8.8	0	0.0	0	0.0	9	10.1	1.000
NOTCH1	8	7.8	1	14.3	0	0.0	7	7.9	0.678
MLH1	8	7.8	1	14.3	0	0.0	7	7.9	0.678
BRAF	7	6.9	0	0.0	0	0.0	7	7.9	1.000
MYC	6	5.9	1	14.3	0	0.0	5	5.6	0.568
APC	5	4.9	0	0.0	0	0.0	5	5.6	1.000
TET2	5	4.9	0	0.0	0	0.0	5	5.6	1.000
BRCA1	4	3.9	0	0.0	2	33.3	2	2.2	0.020
MED12	4	3.9	0	0.0	0	0.0	4	4.5	1.000
MET	4	3.9	0	0.0	0	0.0	4	4.5	1.000
ROS1	4	3.9	0	0.0	0	0.0	4	4.5	1.000
BRCA2	3	2.9	0	0.0	0	0.0	3	3.4	1.000
ARID1A	3	2.9	0	0.0	0	0.0	3	3.4	1.000
CEBPA	3	2.9	0	0.0	1	16.7	2	2.2	0.179
CCND1	3	2.9	0	0.0	0	0.0	3	3.4	1.000
KIT	3	2.9	0	0.0	0	0.0	3	3.4	1.000
MCL1	3	2.9	0	0.0	0	0.0	3	3.4	1.000
AKT1	2	2.0	0	0.0	0	0.0	2	2.2	1.000
CDKN2A	2	2.0	0	0.0	0	0.0	2	2.2	1.000
KDR	2	2.0	0	0.0	0	0.0	2	2.2	1.000
PTEN	2	2.0	0	0.0	1	16.7	1	1.1	0.119
DNMT3A	2	2.0	0	0.0	0	0.0	2	2.2	1.000
JAK3	2	2.0	0	0.0	0	0.0	1	1.1	1.000
STK11	2	2.0	0	0.0	0	0.0	2	2.2	1.000
ARAF	1	1.0	0	0.0	0	0.0	1	1.1	1.000
CALR	1	1.0	0	0.0	0	0.0	1	1.1	1.000
CDH1	1	1.0	0	0.0	0	0.0	1	1.1	1.000
LRP1B	1	1.0	0	0.0	0	0.0	1	1.1	1.000
RAF1	1	1.0	1	14.3	0	0.0	0	0.0	0.127
SCN11A	1	1.0	0	0.0	0	0.0	1	1.1	1.000
SMO	1	1.0	0	0.0	0	0.0	1	1.1	1.000
USP6	1	1.0	1	14.3	0	0.0	0	0.0	0.127

MTOR	1	1.0	0	0.0	0	0.0	1	1.1	1.000
CCNE1	1	1.0	0	0.0	0	0.0	1	1.1	1.000
FGFR4	1	1.0	0	0.0	0	0.0	1	1.1	1.000
ERBB2	1	1.0	0	0.0	0	0.0	1	1.1	1.000
CD274	1	1.0	0	0.0	0	0.0	1	1.1	1.000
CDK4	1	1.0	1	14.3	0	0.0	0	0.0	0.127
CSF1R	1	1.0	0	0.0	0	0.0	1	1.1	1.000
CTNNB1	1	1.0	0	0.0	0	0.0	1	1.1	1.000
ESR1	1	1.0	0	0.0	0	0.0	1	1.1	1.000
FBXW7	1	1.0	0	0.0	0	0.0	1	1.1	0.127
FGFR1	1	1.0	0	0.0	0	0.0	1	1.1	1.000
IRF4	1	1.0	0	0.0	0	0.0	1	1.1	1.000
MAP2K1	1	1.0	0	0.0	0	0.0	1	1.1	1.000
MAP2K2	1	1.0	0	0.0	0	0.0	1	1.1	1.000
MYD88	1	1.0	0	0.0	0	0.0	1	1.1	1.000
MYH13	1	1.0	0	0.0	0	0.0	1	1.1	1.000
NFE2L2	1	1.0	0	0.0	0	0.0	1	1.1	1.000
NPM1	1	1.0	0	0.0	1	16.7	0	0.0	0.059
PARP4	1	1.0	0	0.0	0	0.0	1	1.1	1.000
RAD51B	1	1.0	0	0.0	0	0.0	1	1.1	1.000
SPOP	1	1.0	0	0.0	0	0.0	1	1.1	1.000
TRAF3	1	1.0	0	0.0	0	0.0	1	1.1	1.000
MDM4	1	1.0	0	0.0	0	0.0	1	1.1	1.000
RB1	1	1.0	0	0.0	0	0.0	1	1.1	1.000
FLT3	1	1.0	0	0.0	0	0.0	1	1.1	1.000
FOXP1	1	1.0	0	0.0	0	0.0	1	1.1	1.000
FGFR3	1	1.0	0	0.0	0	0.0	1	1.1	1.000
NKX3-1	1	1.0	0	0.0	0	0.0	1	1.1	1.000

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Supplemental Data Table S6. Simple correlation analysis between recurrent ctDNA gene alterations and clinicopathologic features

The contents of **Supplemental Data** Table 6 are provided in a separate Excel file.

Supplemental Data Table S7. Multivariate analysis to explore genetic factors associated with clinicopathologic features.

Clinical feature	Variable	Univariate	Multivariate	
		P	***P***	**OR (95% CI)**
Hypercalcemia	ISS stage (I *vs*. II *vs.* III)	2.2×10^{-4}	1.000	1.7 (0–∞)
	β2-microglobulin (<5.5 *vs*. ≥5.5)	2.2×10^{-4}	0.998	2.1×10^{14} (0–∞)
	Renal insufficiency (no *vs*. yes)	3.3E-05	0.246	4.3 (0.4–50.7)
	Type of myeloma (IgG *vs*. LCD *vs*. other heavy chain-type)	0.031	0.767	1.0 (0.7–1.3)
	TET2 (wild type *vs*. alteration)	0.048	0.997	9.4×10^{7} (0–∞)
Renal insufficiency	ISS stage (I *vs*. II *vs*. III)	8.8×10^{-9}	1.000	1.0 (0–∞)
	β2-microglobulin (<5.5 *vs*. ≥5.5)	8.8×10^{-9}	0.999	1.0×10^{9} (0–∞)
	Hypercalcemia (no *vs*. yes)	3.3×10^{-5}	0.093	7.9 (0.7–87.0)
	Type of myeloma (IgG *vs*. LCD *vs*. other heavy chain-type)	0.012	0.998	1.0 (0.8–1.3)
	NRAS (wild type *vs*. alteration)	0.031	0.293	4.0 (0.3–52.2)
Paramedullary myeloma	Myeloma-extended sites (no *vs*. yes)	2.0×10^{-14}	4.0×10^{-6}	268.2 (25.1–2,867.0)
	Extramedullary myeloma (no *vs*. yes)	0.025	0.665	0.6 (0.1–4.7)
	TP53 (wild type *vs*. alteration)	0.030	0.217	5.8 (0.4–92.6)
Extramedullary myeloma	Paramedullary myeloma (no *vs*. yes)	0.025	0.441	0.3 (0.0–6.7)
	Cytogenetic status (standard risk *vs*. high risk)	0.040	0.993	1.4×10^{22} (0–∞)

Myeloma-extended sites (no *vs*. yes)	0.001	0.993	7.2×10^{21} (0–∞)
β2-microglobulin (<5.5 *vs*. ≥5.5)	0.029	0.994	1.1×10^{14} (0–∞)
NRAS (wild type *vs*. alteration)	0.032	0.994	6.0×10^{15} (0–∞)

Abbreviations: OR, odds ratio; CI, confidence interval; ISS, International Staging System; LCD, light chain disease.

Supplemental Data Table S8: Prognostic factors for overall and progression-free survival.

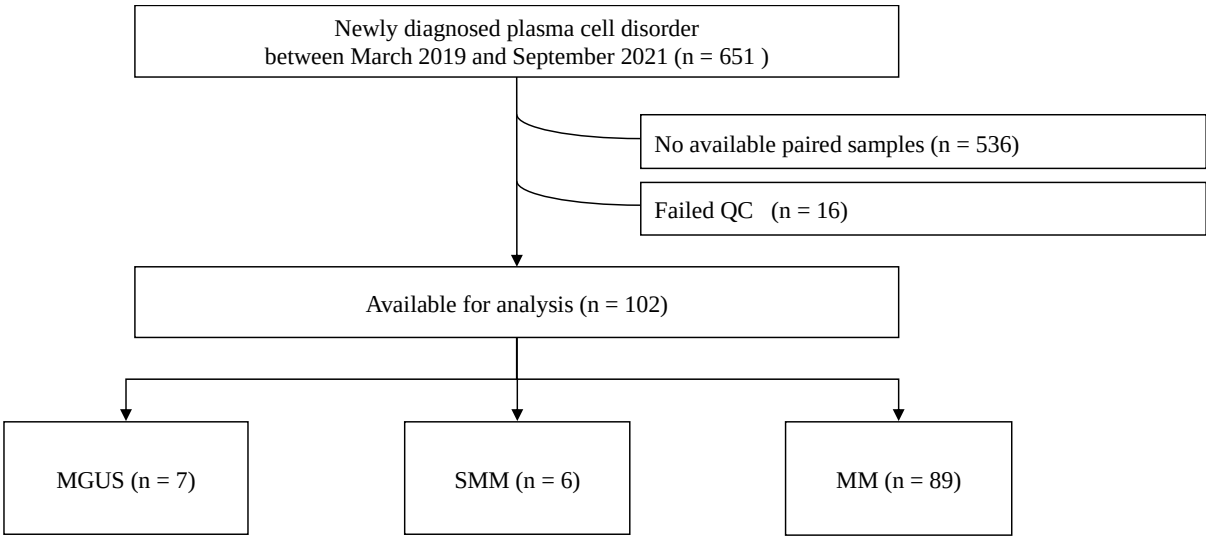
Variable	Overall survival % at 24 months (95% CI)	*P**	Progression-free survival % at 24 months (95% CI)	*P**
Total patients (N=84)	89.5% (82.8–96.7)		54.9% (44.9-67.1)	
Gene alteration (no *vs.* yes)				
KRAS (no, N=69 *vs*. yes, N=15)	86.9% (78.8–95.9) *vs*. 100%	0.268	52.6% (41.7–66.4) *vs*. 65.5% (44.9–95.4)	0.670
TP53 (no, N=74 *vs*. yes, N=10)	90.8% (84.0–98.2) *vs*. 80.0% (58.7–100)	0.225	52.9% (42.3–66.1) *vs*. 70.0% (46.7–100)	0.517
GNAS (no, N=76 *vs*. yes, N=8)	88.3% (80.9–96.3) *vs*. 100%	0.239	54.2% (43.8–67.2) *vs*. 62.5% (36.5–100)	0.716
NIPA2 (no, N=74 *vs*. yes, N=10)	92.3% (88.0–99.1) *vs*. 70.0% (46.7–100)	**0.009**	57.0% (46.4–70.1) *vs*. 40.0% (18.7–85.5)	0.094
ZFHX3 (no, N=77 *vs*. yes, N=7)	89.9% (83.0–97.3) *vs*. 85.7% (63.3–100)	0.312	53.5% (43.1–66.4) *vs*. 71.4% (44.7–100)	0.557
NOTCH1 (no, N=77 *vs*. yes, N=7)	88.4% (81.1–96.4) *vs*. 100%	0.279	53.4% (42.9–66.3) *vs*. 71.4% (44.7–100)	0.400
NRAS (no, N=75 *vs*. yes, N=9)	92.5% (86.3–99.1) *vs*. 62.5% (36.5–100)	**0.030**	58.2% (58.2–47.8) *vs*. 25.0% (7.5–83.0)	**0.035**
MLH1 (no, N=78 *vs*. yes, N=6)	88.6% (81.4–96.4) *vs*. 100	0.324	54.0% (43.7–66.8) *vs*. 66.7% (37.9–100)	0.288
BRAF (no, N=77 *vs*. yes, N=7)	88.4% (81.0–96.4) *vs*. 100%	0.414	56.0% (45.6–68.8) *vs*. 42.9% (18.2–100)	0.454
APC (no, N=79 *vs*. yes, N=5)	91.5% (85.1–98.3) *vs*. 60.0% (29.3–100)	**0.006**	55.9% (45.6–68.5) *vs*. 40.0% (13.7–100)	0.248
TET2 (no, N=79 *vs*. yes, N=5)	91.5% (85.2–98.3) *vs*. 60.0% (29.3–100)	**0.011**	58.5% (48.3–71.0) *vs*. 0%	**<0.001**
MYC (no, N=80 *vs*. yes, N=4)	90.2% (83.5–97.4) *vs*. 75.0% (42.6–100)	0.502	55.1% (44.9–67.7) *vs*. 50.0% (18.8–100)	0.990
Clinical variables				
Sex (male, N=41 *vs*. female, N=43)	89.6% (80.4–99.9) *vs*. 89.4% (80.0–99.8)	0.555	52.3% (38.8–70.6) *vs*. 57.8% (44.4–75.4)	0.761
Age (<65 yrs, N=37 *vs*. ≥65 yrs, N=47)	91.8% (83.3–100) *vs*. 87.2% (77.3–98.5)	0.097	69.3% (55.7–86.2) *vs*. 43.0% (30.4–60.9)	**0.004**

Bone lytic lesion (no, N=21 *vs*. yes, N=63)	90.2% (78.2–100) *vs*. 89.0% (81.0–97.8)	0.490	54.8% (36.7–81.9) *vs*. 55.1% (43.7–69.3)	0.898
Renal insufficiency (no, N=72 *vs*. yes, N=12)	90.6% (83.7–98.1) *vs*. 81.8% (61.9–100)	0.786	57.9% (47.2–70.9) *vs*. 36.4% (16.6–79.5)	0.186
Anemia (no, N=30 *vs*. yes, N=54)	85.9% (74.0–99.7) *vs*. 91.5% (83.7–99.9)	0.801	54.4% (38.7–76.3) *vs*. 55.4% (43.3–71.0)	0.746
Hypercalcemia (no, N=79 *vs*. yes, N=5)	90.1% (83.4–97.4) *vs*. 80.0% (51.6–100)	0.594	55.9% (45.7–68.5) *vs*. 40.0% (13.7–100)	0.650
Type of myeloma (LCD, N=17 *vs*. IgG, N=43 *vs*. others, N=24)	87.8% (73.4–100) *vs*. 88.3% (77.9–100) *vs*. 91.7% (81.3–100)	0.827	46.3% (27.6–77.8) *vs*. 65.0% (51.8–81.7) *vs*. 43.8% (27.3–70.2)	0.285
Light chain-type (kappa, N=49 *vs*. lambda, N=35)	93.3% (86.3–100) *vs*. 84.5% (72.8–98.1)	0.065	63.1% (50.5–78.9) *vs*. 44.3% (30.4–64.7)	**0.037**
Myeloma-extended sites (no, N=57 *vs*. yes, N=27)	92.7% (86.0–99.9) *vs*. 83.0% (69.1–99.8)	0.646	61.3% (49.5–75.8) *vs*. 41.6% (26.1–66.3)	0.098
Paramedullary myeloma (no, N=62 *vs*. yes, N=22)	93.3% (87.2–99.9) *vs*. 78.6% (61.9–99.8)	0.340	56.8% (45.5–70.9) *vs*. 49.7% (31.7–77.7)	0.536
Extramedullary myeloma (no, N=76 *vs*. yes, N=8)	91.2% (84.6–98.2) *vs*. 71.4% (44.7–100)	0.147	56.1% (45.7–68.8) *vs*. 42.9% (18.2–100)	0.551
ISS (I, N=31 *vs*. II, N=29 vs. III, N=21)	82.2% (69.0–97.9) *vs*. 100% *vs*. 85.0% (70.7–100)	0.921	56.9% (41.5–77.9) *vs*. 59.1% (43.0–81.2) vs. 45.0% (27.7–73.1)	0.476
Cytogenetic profile (standard risk, N=29 vs. high risk, N=53)	89.4% (78.7–100.0) *vs*. 89.2% (80.7–98.7)	0.67	75.2% (60.8–93.0) *vs*. 45.0% (33.1–61.0)	**0.01**
β2-Microglobulin (<5.5, N=60 *vs*. ≥5.5	90.4% (82.7–98.9) *vs*. 85.0% (70.7–100)	0.988	57.9% (46.3–72.4) *vs*. 45.0% (27.7–73.1)	0.243

μg/mL, N=21)				
Albumin (<3.5, N=48 *vs*. ≥3.5 g/dl, N=36)	88.3% (79.1–98.7) *vs*. 90.8% (81.4–100)	0.677	53.0% (40.4–69.7) *vs*. 57.4% (42.6–77.2)	0.484
Lactate dehydrogenase (normal range, N=66 *vs*. ≥upper limit of normal, N=18)	93.7% (87.8–99.9) *vs*. 74.5% (55.7–99.6)	**0.020**	57.2% (46.1–70.9) *vs*. 47.4% (28.7–78.2)	0.412
Absolute neutrophil count (<0.8, N=3 *vs*. ≥0.8×10^9/L, N=81)	100% *vs*. 89.1% (82.1–96.6)	0.452	66.7% (30.0–100) *vs*. 54.5% (44.4–67.0)	0.515
Platelet count (<1.0, N=9 *vs*. ≥1.0×10^9/L, N=75)	87.5% (67.3–100) *vs*. 89.6% (82.5–97.3)	0.159	38.1% (15.7–92.4) *vs*. 56.6% (46.1–69.5)	0.741
Transplant eligibility (ineligible, N=36 *vs*. eligible, N=48)	83.3% (70.9–97.9) *vs*. 93.7% (87.0–100)	**0.007**	25.3% (13.9–46.2) *vs*. 76.2% (64.8–89.6)	**<0.001**
Treatment regimen (doublet, N=13 *vs*. triplet, N=65 *vs*. quadruplet, N=6)	79.5% (57.7–100) *vs*. 90.3% (83.3–98.0) *vs*. 100%	0.054	33.8% (15.3–74.9) *vs*. 56.7% (45.6–70.5) *vs*. 83.3% (58.3–100)	**0.026**

*Univariate survival analysis was performed using the Kaplan–Meier method. Differences in survival curves were assessed with the log-rank test.

Abbreviations: CI, confidence interval; LCD, light chain disease; ISS, International Staging System.



Supplemental Data Fig. S1. Flow diagram of cohort recruitment. The cohort consisted of 102 patients who were diagnosed as having monoclonal gammopathy of undetermined significance (MGUS) (N=7), smoldering multiple myeloma (SMM) (N=6), or multiple myeloma (MM) (N=89).

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